Boric acid and carbolic acid are the only antiseptics I have used frequently; the results have led me to think that the former, or both together, give all we want, and as I believe that a great part of the benefit comes from the cleansing and complete emptying of the rectum, the least irritating and least dangerous germicide ought to be preferred.

A BACTERIOLOGICAL STUDY OF DRINKING-WATER.[1]

By Victor C. Vaughan, Ph.D., M.D.,
of Ann Arbor, Michigan.

Since the opening of the Hygienic Laboratory of Michigan University, October 1, 1888, a large portion of my time, devoted to laboratory work, has been given to the bacteriological study of drinking water. While I have no great discovery to announce to you, I have thought that a brief record of the results obtained after three and one-half years of work might not be wholly devoid of interest. Health officers are given the privilege of sending samples of food and drink to the laboratory for examination, where the work is done for a small fee, which is supposed to cover the cost of material. I have been anxious from the first that this work should be of practical benefit to the people for whom it has been done, and should also constitute a contribution to science.

Having had, before beginning this work, some years of experience in the chemical analysis of drinking-water, and having been convinced of the inadequacy of the evidence furnished by the same, I have entrusted that part of the work, in most instances, to trained assistants, and I am sure that whatever its value may be, the work has been well done. Drs. F. W. Brewer and A. Ives have made most of the chemical analyses, which will be reported in part later in this paper. A small number of the chemical examinations, and all of the bacteriological work, has been done by myself, and I alone am responsible for any errors which may be detected in the same.

We keep at the laboratory a number of sterilized bottles with glass stoppers, and of two and one-half litres capacity. These bottles are sterilized by steam, then set in wooden crates so arranged that the handle of the crate holds the stopper in place, and makes it impossible to open the bottle without removing the handle, which is fastened by means of screws. The use of sealing wax is forbidden, and in cases where the over-zealous collector has resorted to this method "to make things more secure," the samples have been discarded and fresh bottles have been sent, with the request that the sealing be omitted. In some instances a piece of sublimate gauze has been placed over the stopper and fastened about the neck of the bottle. This admits of the collector placing his seal upon the outside of the gauze. A health officer writes or telegraphs that he wishes one or more samples examined, and the desired number of bottles is sent. He is instructed to fill them and to return them immediately. In the great majority of cases the bacteriological examination has been begun at the laboratory within thirty-six hours after the samples have been collected. In some instances I have instructed the collector to obtain new bottles and to sterilize them, but as a rule this is not satisfactory, and many samples have been discarded on learning the method of sterilization employed; the above-described method is now exclusively used.

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[1] Read before the Association of American Physicians at Washington, May, 1892.

Immediately on receipt of the water at the laboratory, gelatin plates are made, and at the same time from two to six beef-tea tubes are inoculated with the water, and placed in an incubator at 38° C. The temperature at which these beef-tea tubes are kept is, as we shall see, a matter of importance. The amount of water added to the gelatin and beef-tea tubes has varied from one drop to one cubic centimetre. My experience has led me to prefer from three to six gelatin plates, each made with one drop of water, to one plate made with one cubic centimetre, provided that in the making of each plate a drop of water is taken up in a fresh pipette after shaking the water in the bottle. I am convinced that in this manner one is less likely to fail to get on the plates all the germs in the sample of water.

After the beef-tea tubes have stood in the incubator at 38° for twenty-four hours, it is observed whether or not any germs have developed, and a record is made of this observation. As we shall see further on, the greater number of the germs found in drinking-water do not multiply at all, or do so very feebly, at 38° C. Whether the germs have developed or not, animals are inoculated with the beef-tea cultures. If certain germs be present, some of the animals thus inoculated will die within a period of time which varies with the animal employed and the method of inoculation. Practically I have used in my routine work, in the majority of instances, white rats. The reasons for selecting this animal are the following: 1. The rat is easily kept and multiplies rapidly, and where one uses from three to four hundred animals in a year, this becomes a matter of some importance. If the animal does not die, it is not used in testing other samples of water, because of the fear that a tolerance for certain water germs may have been established. Some special experiments, however, recently made to test this point seem to show that I have overestimated this danger. 2. As compared with mice, and even with guinea-pigs and rabbits, the operation of inoculation *per se* has less effect upon the rat.

In the routine work, I have usually inoculated the rat by the injection of from one to two cubic centimetres of the beef-tea culture into the abdominal cavity. The reasons for selecting this method of inoculation are: 1. The greater certainty of killing, providing a toxicogenic germ is present, and, 2. the shortening of the length of time between the inoculation and death. These very reasons, however, which have led me to select this animal and the mode of inoculation in making the first rough test of the water, have also induced me to repeat the inoculations, using other animals and other methods of inoculation, in all cases where the results obtained by the routine tests were positive. I will give later on in detail the results of some of these inoculations.

If the animals die, plates are made from the spleen, liver, and kidney in *all* instances, and from the blood in some. The germs which develop on the plates made from these organs are compared with those on the plates made directly from the water, and tube cultures are made of all germs. In the majority of examinations each germ has been studied sufficiently to enable me to identify it. The number of germs on the plates made directly from the water are counted after twenty-four, forty-eight, and seventy-two hours, and as some plates have always been made with one drop of the water, the number on these is given in the table.

I have used the word "toxicogenic" in referring to those germs found in drinking-water which prove fatal to inoculated animals. I have also elsewhere employed the same term in referring to certain other germs. The word toxicogenic (poison-producing) explains itself, but it may be asked why I have taken the liberty of coining such a word, and exactly what meaning do I attach to it? How does it differ in meaning from pathogenic? I suppose that all now believe that germs which are harmful when introduced into the bodies of men and the lower animals owe their deleterious effects to certain chemical products which they elaborate. Therefore, all pathogenic germs are toxicogenic, but are all toxicogenic germs pathogenic? The answer to this question depends upon the meaning which one attaches to the word pathogenic. I prefer to use this word in the sense indicated by its derivation, *i. e.*, as disease-producing. Now, there are poison-producing germs which are fatal to man and some of the lower animals which are not known to induce any characteristic symptoms or any specific lesions. They grow and multiply in the body, sometimes with fearful rapidity, and prove speedily fatal. Such are the germs which induce the great variety of symptoms which we designate as the summer diarrhœas of infancy, and which would more properly be called milk-poisoning. In the same class are those germs which cause serious results to follow the eating of sausage, cheese, canned salmon, lobster, etc. These germs, it seems to me, should not be said to be pathogenic, but they certainly are toxicogenic. Now, I find in drinking-water a number of germs differing from one another morphologically and in their growth on various nutritive media, which do not, in the animals upon which I have experimented, induce any specific disease which can be recognized either by symptoms during life or by lesions after death. These germs I call toxicogenic. A germ, in order to be toxicogenic to a given animal, must multiply in the body of the animal and there elaborate its poison. In this way a germ may be toxicogenic to one animal and not to another.

We must distinguish between the germs which kill by virtue of the chemical poisons which they produce outside the body and those which produce chemical poisons within the body. This difference may be well illustrated by some of the water germs. If we allow the white liquefying bacillus of water to grow in beef-tea for some days at the ordinary temperature of a room, and then inject three or four cubic centimetres of the culture into the abdominal cavity of a white rat, the animal will succumb within a few hours. But this bacillus will not multiply at the temperature of the rat's body, and the fatal effects are due solely to the chemical poisons which the germ produced in the beef-tea before the inoculation. The truth of this may be demonstrated by filtering the culture through a Chamberland filter, and it will be found that the same amount of the germ-free filtrate will kill. I would not say that this germ is toxicogenic to the rat, and I have been careful in all of my experiments to avoid mistaking such a germ for those which I, in this paper, designate as toxicogenic germs. In the routine work this danger is avoided by keeping my beef-tea culture in an incubator the temperature of which will not fall below 38° C. Moreover, in order to satisfy myself that even when the germ is grown at this temperature, the fatal effects are not due to poisons generated in the tube, I have injected cultures which have been freed from germs by filtration through porcelain, and have found that six times the fatal quantity of the germ-containing cultures may be injected without fatal effect. Thus one cubic centimetre of a beef-tea culture of bacillus venenosus twenty-four hours old, invariably kills white rats, while six cubic centimetres of the same culture after filtration fails to cause death. It is true that a small amount of poison is produced in the tube, and this probably has some influence in slightly lowering the vitality of the animal, while the germ rapidly multiplies and produces a sufficient quantity of poison to cause death.

With the method of work now understood, I will give the conclusions which I have reached concerning the value of a bacteriological examination of water, and then will close with tabular statements of the investigations upon which these conclusions are based.

Some of the conclusions which have been reached in this study are as follows:

1. Many of the germs found in drinking-water will not grow at the

temperature of the human body. These germs, therefore, are not capable of inducing disease. It matters not how rich a given sample of water may be in these germs, if it contains no others it cannot be said that the water is a source of typhoid fever. The freedom from typhoid fever of communities using such waters seems to justify this conclusion. Such a water may not be, and certainly often is not, a desirable drinking-water. It may be turbid with suspended matter, unpleasant to the taste, and give off a disgusting odor, but there is no evidence that it can cause disease. Several interesting examples of this kind have come under observation. One of these may be mentioned. A certain city of about ten thousand inhabitants took for awhile its public water supply from a shallow lake, the bottom of which was covered with decomposing organic matter of vegetable origin. This water was turbid, unpleasant to the taste, and gave off unpleasant odors, but none of the bacteria in it grew at 38° C. On the other hand, many of the inhabitants of the city took their drinking-water from shallow wells, the water of which was clear, sparkling, and palatable. The well water, however, contained germs which grew abundantly at 38° C., and which were toxicogenic to animals. The use of the lake water in preference to that supplied by the wells was recommended early in the spring of 1891. Some followed the advice, others did not. During the summer and early fall of 1891 there were more than two hundred cases of typhoid fever in the city, and at a public meeting held by the State Board of Health in this place, about the last of October, every physician in the city agreed to the statement that there had not been a case of typhoid fever among those who had used the lake water exclusively. This positive testimony of the physicians was also confirmed by other citizens.

2. Of the germs which grow at 38° C. or at higher temperatures, some are fatal to animals when injected subcutaneously, while others are not. This renders a division of them into toxicogenic and non-toxicogenic germs possible.

3. There is no proof that these non-toxicogenic germs can multiply in the animal body. Indeed, all the evidence which has been gathered so far goes to show that they not only fail to multiply in the bodies of the rat, mouse, guinea-pig, and rabbit, but that they soon die when injected under the skin or into the abdomen. However, this does not furnish positive evidence that they would not multiply in the body of man. For these reasons waters containing these germs have not been positively condemned, though it has been advised in some cases that their use should be discontinued.

4. Some of the toxicogenic germs found in drinking-water produce the same symptoms and the same post-mortem appearances in the above-mentioned animals as are observed after the similar employment of

Eberth's germs. Moreover, their toxicogenic properties, as tested upon these animals, are fully equal to those of Eberth's germs. They will not only live, but will multiply in the animal body. Waters containing these germs have in all cases been condemned.

5. More than one germ obtained from drinking-water grows with an invisible growth on potato.

6. Several germs found in drinking-water will grow on the media proposed by Parietti, Uffelmann, and others, as means of recognition of the Eberth germ.

The results of the examination of one hundred and forty-eight waters are given in the table. The waters are divided into three classes. The first contains those which are supposed to be causing typhoid fever at the time the water is sent, or to have caused the disease prevailing at the time. In other words, the water has been sent on account of the present existence of typhoid fever among those using it. In the second class are these waters which are not supposed to be causing typhoid fever at the time, but which are suspicious on account of unsanitary surroundings, or on account of previous sickness among those drinking it. In the third class are given those waters which have not been suspected of causing sickness, and the sanitary surroundings of which are not said to be especially bad. Several of the third class have been sent for analysis in view of their possible selection for the water supply of cities and villages. The figures given in the chemical part of the table represent parts per million, the chlorine being calculated as sodium chloride. The chemical analyses are much more extensive than shown in the table. I have given the results of the estimation of free and albuminoid ammonia and chlorine, because much importance has generally been attached to these by chemists. In addition to the figures given in the table a record is made of the physical properties, hardness, amount of residue organic and inorganic, sulphates, earthy bases, nitrates, nitrites, and amount of oxygen consumed in the permanganate process. The number of germs in one drop of the water is given. In the column "kind of germ," the figures refer to the corresponding ones given in the descriptive tables of the individual germ, found later on. The germs which are indicated by the letters C, D, and E, are not given in the descriptive tables, because the cultures have been lost through negligence in not transplanting as often as should have been done. I have elsewhere[1] described these germs, and the following is a brief description of them:

Bacillus C rapidly liquefies gelatine, the liquefaction beginning at the surface. It forms gas, is stained by the ordinary agents and by Gram's method. It resembles Eberth's germ in the formation of an invisible growth on potato, but in no other respect. Some twenty rats were killed with this germ. The minimum fatal amount of the twenty-four hours' culture for white rats seemed to be between ten and fifteen drops. One strange observation was made. Cultures nine or ten days old are apparently less virulent than those only twenty-four hours old. Moreover, the germ decreases in virulence as it is grown in the artificial media, until in about the sixth generation it may be employed in four times the quantity first used without any effect. The post-mortem appearances are quite different from those observed with Nos. 29 and 31, as given in the descriptive tables in this paper. The intestines are reddened, but the liver and spleen are tarry black. Cultures from these organs show numerous liquefying colonies.

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[1] The Medical News, June 14, 1890.

Bacillus D is probably not pathogenic, but kills on account of the chemical poisons rapidly developed. Further study of this germ is needed before extended statements can be made concerning it. I can only say that it does not correspond with any germ given in the tables of Eisenberg.

Bacillus E is non-liquefying. It grows slowly in stick cultures, and forms a scarcely visible growth on potato. It decreases in virulence with artificial cultivation, and while none of the other germs in these waters showed any pathogenic properties in pure cultures, beef-tea inoculated with a drop of the water never failed to kill rabbits and rats, while bacillus E in pure cultures frequently failed to produce death. Moreover, after the death of these animals inoculated with the mixed culture, bacillus E was the only germ found living in the organs. The following quotation from my notes will illustrate this: October 28, 1889, a large rabbit was inoculated with sixty drops of beef-tea, to which one drop of the Negaunee public water had been added. October 21, animal found dead at 9 A. M. Post-mortem showed small intestines and mesentery highly inflamed; no other changes observable. Plates made from spleen, liver, and kidney showed pure culture of germ marked "Negaunee." It is possible that this is Eberth's germ, though of this I am not certain. Unless this be the exception, I have not found Eberth's germ in any of the waters.

The waters No. 140 to No. 147, inclusive, I have not been able to classify. When these samples were sent I preferred not to know the source of each water, and asked that they be designated by numbers. Since sending in my report I have twice written asking that I may be informed of the source of each water, but my request has not been answered. Omitting these samples, the remaining one hundred and forty samples are divided into the following classes, according to their supposed relation to typhoid fever. Thirty-three are placed in the first class, *i. e.*, they are waters which were believed to be causing typhoid fever at the time the samples were sent. Forty-four belong to the second class, *i. e.*, while typhoid fever is not prevailing among those who are using the water, the water is regarded as suspicious on account of the former existence of typhoid fever or on account of unsanitary surroundings. Sixty-three belong to the third class. The first sixteen waters of first class I have elsewhere[1] discussed. In fifteen of these germs toxicogenic to the lower animals were found.

No. 90 in the next belonging to this class: The city of Sault Ste. Marie takes its water supply above the rapids, and ordinarily it is a very pure water. In the fall of 1890, owing to a break in the lock, more than two hundred boats were detained for two days above the lock and in the immediate vicinity of the intake. Some ten days later typhoid fever appeared, and the deaths were reported to be not less than thirty. All the cases appeared within a few weeks, and sample No. 90 was taken at this time. After this first outbreak there were no new cases. Samples 103, 104, and 105 were collected about one month later, and while the disease was still prevailing, but at a time when no new cases were being reported. I have, therefore, placed them in the second class.

No. 92 furnishes a lesson of interest. Typhoid fever was confined to a certain portion of the city. The supply-pipe carrying water to this portion was, I am told, a wooden one. At one point of its course a privy vault had been dug down upon the pipe, and had been used for some years. The wooden pipe, much decayed, was covered in the vault with fecal matter.

Dr. E. P. Christian, of Wyandotte, Mich., sent me samples Nos. 93 and 94, and along with these a sample of milk. The doctor had about thirty cases of continued fever, some of which he designated as typical and others as atypical typhoid. He soon ascertained that the families in which the typical cases existed took their milk from the same dairyman. No. 93 was taken from the well of the dairyman, and in both this and the milk I found bacillus venenosus. In the river water I found the liquefying toxicogenic bacillus. All who had the fever had used the river water, and some of those who drank both the milk and the river water had had the disease in an atypical form, but none of those who did not drink the milk had the typical form of the disease. These observations were reported to me by Dr. Christian after I sent him the report of the examination. My conclusions, as stated in the report, were as follows:

"I regard both the waters and the milk as unsafe. Of the two waters that from the well is the worst both chemically and bacteriologically. The milk was examined only bacteriologically. The poison-producing germ in the well water is identical with that in the milk. I am inclined to the opinion that if the typhoid fever is due to one of the waters, the well water is the one most likely to be the cause. Eberth's germ (the so-called typhoid fever germ) was not found in any of the samples. This, however, does not mean, in my opinion, that the waters would not cause typhoid fever. I have never yet found Eberth's germ in drinking-water (with one possible exception). The river water probably has changed in character since the sample was taken, but the well water certainly should not be used."

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[1] The Medical News, June 14, 1890.

The men who had typhoid fever at Torch Lake worked in a saw mill, which was located on the bank of the lake. The well from which sample No. 95 was taken was at the mill, and near both well and lake was a large privy vault used by the men while at work. Sample No. 96 was taken from a well which was used by these men when not at work, and was more than a quarter of a mile from both well (No. 95) and lake. I knew nothing of these facts until some time after I had reported, as the samples when sent to me were distinguished by numbers.

In Nos. 101 and 102 I could find no toxicogenic germ, although it was believed that these waters were at the time causing typhoid fever. No. 102 was taken from a farmer's well, and one member of the family after another came down with the fever. Whether there were other possible sources of infection about the place or not I do not know.

Sample No. 107 furnishes another illustration of my failure to find a toxicogenic germ in a water of the first class.

Nos. 109 and 110 were from wells from both of which the sick men had been accustomed to drink before coming down with the fever.

Nos. 111, 112, 113, and 114 came from the public water supply of Duluth, in January, 1891, during the prevalence of a very severe epidemic of typhoid fever. I found a toxicogenic germ in only one of the samples. This sample was taken, so the health officer informed me, from a tap in a doctor's office, the occupant of which had gone home some two weeks before with typhoid fever, and, so far as known, the tap had not been used since that time.

Every now and then some one announces a new test, by means of which the Eberth germ can be distinguished from other germs in water. These announcements are founded upon comparisons with a few water germs, or with those which happen to exist in one or two samples of water. None of these tests have been systematically tried with a large number of germs.

One of these tests, proposed by Parietti,[1] is made as follows: To 10 c.c. of neutral bouillon there are added from three to nine drops of the following solution:

Carbolic acid	5 grammes.
Pure hydrochloric acid	4 "
Distilled water	100 "

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[1] "Metodo di ricerca del Bacillo del Tifo nelle acque potabili." Rivista d'Igiene e Sanità Pubblica, 1890.

SAMPLES OF DRINKING-WATER EXAMINED IN THE HYGIENIC LABORATORY OF MICHIGAN UNIVERSITY.

From October 1, 1888, to May 1, 1890.

No.	Water sent from	Source.	Class.	Free ammonia.	Albuminoid ammonia.	Chlorine.	Number of germs.	Kind of germs.	Remarks.
1	Omaha, Nebraska,	Well	Second	0.168	0.118	129.0	50	No. 13 was found in all the Omaha samples. No. 10 in samples No. 4. No. 1 in Nos. 1, 3, and 4. Nos. 11 and 12 in samples 5, 7, 8, 9, and 10. Undescribed germs were found in samples 2 and 4.	Pure cultures of each of these germs in beef-tea, twenty four hours old, were injected into the abdominal cavity of rabbits and rats. All were without effect.
2	" "	"	"	0.050	0.124	97.0	46		
3	" "	"	"	0.064	0.230	12.0	120		
4	" "	"	"	0.102	0.110	45.0	50		
5	" "	"	"	0.094	0.112	23.0	26		
6	" "	"	"	0.028	0.090	31.0	18		
7	" "	"	"	0.022	0.102	48.0	38		
8	" "	"	"	0.172	0.088	48.0	36		
9	" "	"	"	0.054	0.068	98.0	20		
10	" "	"	"	0.032	0.096	8.0	8		
11	Coldwater, Mich.	"	"	0.270	0.015	8.0	500	Nos. 10, 13, and 16 were found in these waters.	Inoculations of rabbits were without effect.
12	" "	"	"	0.015	0.025	14.0	712		
13	" "	"	"	0.270	0.040	15.0	370		
14	" "	"	"	0.025	0.040	12.0	580		
15	Ida, Mich.	"	"	0.910	0.120	155.0	2000	No. 13	Inoculation of rabbit was without effect.
16	Brown City, Mich.	"	"	0.220	0.096	8.0	400	Nos. 10 and 13.	Inoculations of rabbits were without effect.
17	Kalamazoo, Mich.	"	Third	0.041	0.084	7.0	63	Nos. 10 and 13	" " " " " "
18	" "	"	"	0.020	0.056	7.0	60		
19	Mount Pleasant, Mich.	River	Second	0.094	0.208	79.0	3000	Nos. 10 and 13	Inoculations of rats were without effect.
20	Gambier, Ohio,	Well	"	0.065	0.062	210.0	683	Nos. 1, 10, 11, 12, 13, and 16	Inoculations of all the germs on rats and rabbits were without effect.
21	Allegan, Mich.	"	"	0.206	0.148	33.0	300	No. 13.	Inoculation of rabbit was without effect.
22	Three Rivers, Mich.	"	Third	0.070	0.046	9.0	25	No. 13	Inoculations of rats and rabbits were without effect.
23	" " "	"	"	0.108	0.072	7.0	20		
24	" " "	"	"	0.024	0.054	7.0	22		
25	Allegan, Mich.	Hydrant	"	0.220	0.060	31.0	40	No. 13	Inoculations of rats were without effect.
26	" "	Artesian	"	0.272	0.556	32.0	25		
27	Coldwater, Mich.	Well	"	0.024	0.038	14.0	16	No. 13	Inoculation of rat was without effect.
28	Kalamazoo, Mich.	"	"	0.144	0.058	9.0	280	Nos. 10 and 13	Inoculations of rabbits were without effect.
29	Tecumseh, Mich.	"	Second	0.007	0.078	10.0	40	No. 13	Inoculation of rat was without effect.
30	Ishpeming, Mich.	Lake	Third	0.222	0.418	6.0	455	Nos. 10 and 13	Inoculations of rats and rabbits were without effect.
31	" "	"	"	0.036	0.222	2.0	50		
32	" "	"	"	0.038	0.208	3.0	48		
33	Three Rivers, Mich.	Well	"	0.200	0.036	0.9	30	No. 13	Inoculations of rats were without effect.
34	" " "	"	"	0.060	0.102	0.7	25		
35	" " "	"	"	0.016	0.062	1.0	22		

36	St. Louis, Mich.	Reservoir	Third	0.060	0.210	23.0	8400	Nos. 10, 12, 13, 16, and 20	Inoculations of rats and rabbits were without effect.
37	" "	Pine River	"	0.014	0.320	21.0	1008	Nos. 10, 13, and 16	
38	Climax, Mich.	Well	Second	0.008	0.084	4.5	105	Nos. 10 and 13	Inoculations of rats were without effect.
39	Grand Rapids, Mich.	River	Third	0.030	0.134	10.0	36,000	Bacillus D.	Rabbits inoculated with No. 39 died, while those inoculated with No. 40 were not affected.
40	" " "	"	"	0.024	0.075	6.0	120		
41	Ann Arbor, Mich.	Cistern	First	2.223	0.908	3.0	3000	No. 29	Rats, guinea-pigs, and rabbits inoculated with this germ died.
42	Negaunee, Mich.	Lake Teal	"	0.228	0.368	30.0	920	In No. 46, germs Nos. 1, 10, and 13 were found. In Nos. 42, 43, 44, and 45 there was found in addition to the above bacillus E.	Rabbits inoculated with bacillus E died; those inoculated with the other germs from the same waters were not affected.
43	" "	" "	"	0.232	0.460	30.0	1240		
44	" "	" "	"	0.296	0.418	40.0	772		
45	" "	Mine.	"	0.312	0.236	140.0	2060		
46	" "	Sand Shaft	Third	0.012	0.120	40.0	772		
47	Mendon, Mich.	Well	Second	0.019	0.018	4.0	25	No. 13	Rats were not affected.
48	" "	"	"	0.020	0.210	34.0	100	Nos. 1, 10, and 13	
49	Dearfield, Mich.	"	First	0.508	0.158	695.0	898	Bacillus C.	Rats inoculated with this germ died.
50	Northville, Mich.	"	Second	1.136	0.732	6.7	1550	No. 13	Inoculations of rats were without effect.
51	Iron Mountain, Mich.	"	First	1.256	1.920	44.0	1500	Nos. 13 and 31 were found in all these waters.	Rats, guinea-pigs, and rabbits inoculated with No. 31 died.
52	" " "	"	"	0.384	0.840	20.0	1350		
53	" " "	"	"	0.784	0.980	52.0	1260		
54	" " "	"	"	0.904	1.360	16.0	1700		
55	" " "	"	"	0.456	0.944	12.0	850		
56	" " "	"	"	0.464	1.256	28.0	950		
57	Ann Arbor, Mich.	Cistern.	"	0.318	0.262	6.0	1201	No. 29	Rats, guinea-pigs, and rabbits inoculated with No. 29 died.
58	" " "	"	"	0.492	0.504	9.0	1770		
59	Rankine, Illinois	Well	"	0.512	0.332	21.0	738	Nos. 13 and 31	No. 31 is fatal to rats, guinea-pigs, and rabbits.
60	Pomfret, Conn.	"	Second	0.104	0.116	4.0	151	Nos. 10 and 13	Inoculations of rats were without effect.
61	Sherwood, Mich.	"	First	0.194	1.160	7.0	4116	No 14	Rats inoculated with this germ were not affected.
62	Soudan, Minn.	Lake	Third	0.144	0.488	3.0	428	No. 11 and bacillus E.	Rats inoculated with E died.
63	" "	"	"	0.132	0.488	4.0	459	No. 11	Rats inoculated with No. 11 were not affected.
64	" "	Well	Second	0.120	0.099	2.0	28	No. 11	
65	" "	"	"	0.072	0.124	2.0	4	No. 29	Rats inoculated with No. 29 died.
66	Lake City, Mich.	Lake	Third	0.154	0.280	3.0	121	No. 11	Rats were inoculated without effect.
67	Iron Mountain, Mich.	"	"	0.260	0.270	4.5	9	No. 11	" " " " "
68	Ironwood, Mich.	Well	"	0.176	0.218	9.9	12	No. 11	" " " " "
69	Ann Arbor, Mich.	Spring	"	0.012	0.016	2.5	0		
70	" " "	"	"	0.044	0.120	4.0	4	No. 11	" " " " "
71	" " "	"	"	0.050	0.080	6.0	0		
72	" " "	"	"	0.090	0.214	4.0	6	No 11	" " " " "
73	Leslie, Mich.	Well	Second	0.536	0.849	14.0	104	Nos. 11 and 13	" " " " "
74	" "	"	"	1.144	0.984	12.5	2940		
75	Ann Arbor, Mich.	"	Third	0.061	0.064	17.0	150	No. 14	" " " " "
76	" " "	"	"	0.068	0.070	6.0	60	No. 14	" " " " "
77	" " "	Spring	"	0.124	0.194	3.5	295	No. 11	" " " " "

SAMPLES OF DRINKING-WATER EXAMINED IN THE HYGIENIC LABORATORY OF MICHIGAN UNIVERSITY.

From May 1, 1890, to May 1, 1892.

No.	Water sent from	Source.	Class.	Free ammonia.	Albuminoid ammonia.	Chlorine.	Number of germs.	Kind of germs.	Remarks.
78	Three Rivers, Mich.	Well	Third	0.015	0.019	6.288	8 after 72 hours	No. 13	1 c.c. of beef-tea, twenty-four hours old, was injected into the abdominal cavity of rats without effect.
79	East Lake, Mich.	"	"	0.067	0.058	41.71	None	. . . . .	60 drops of beef-tea, twenty-four hours old, were injected into the abdominal cavity of rabbit without effect.
80	Gladwin, Mich.	"	"	0.374	0.011	11.8	63 after 48 hours	Nos. 13 and 14	Inoculation of rat was without effect.
81	Alpena, Mich.	Hydrant	"	0.148	0.37	6.60	22 after 72 hours	Nos. 10 and 13	
82	" "	Spring	"	0.072	0.174	4.61	2880 after 72 hours	Nos. 10 and 13	Inoculations of rats were without effect.
83	Soudan, Minn.	Well	"	0.05	0.156	1.81	6 after 72 hours	No. 14	Inoculation of rat was without effect
84	" "	"	"	0.058	0.132	2.14	3 after 72 hours	Nos. 10 and 13	Inoculations of rats were without effect.
85	" "	"	"	0.076	0.14	2.14	None	. . . . .	Inoculation of rat without effect.
86	Gladwin, Mich.	(Artesian) "	"	0.26	0.102	1.32	10	Unknown	60 drops of beef-tea, twenty-four hours old, injected into abdominal cavity of guinea-pig. Animal died.
87	Imlay City, Mich.	"	"	0.52	0.052	1321.00	98 after 48 hours	Nos. 10 and 13 and unknown	1 drachm of beef-tea, twenty-four hours old, injected into abdominal cavity of a rat. Death in six hours
88	Gladwin, Mich.	"	"	0.526	0.116	21.45	12 after 72 hours	Nos. 10 and 13	20 drops of beef-tea culture, twenty-four years old, injected into abdominal cavity of rat. No effect.
89	Imlay City, Mich.	"	"	0.462	0.102	1328.25	None	. . . . .	Inoculation of rat with negative results.
90	Sault St. Marie, Mich.	Lake	First	0.221	0.168	3.30	2000	No. 28	1 drachm of beef-tea, twenty-four hours old, injected into abdominal cavity of rat produced death.
91	Bay City, Mich.	Hydrant	Third	0.34	0.48	425.70	420	No. 10	Inoculation of rat produced no effect.
92	" " "	"	First	1.00	0.68	465.30	450	No. 31	20 drops of beef-tea, twenty-four hours old, injected into abdominal cavity of rat produced death.
93	Wyandotte, Mich.	Well	"	0.40	0.56	266.4	2430	Nos. 10 and 28	20 drops of beef-tea culture, twenty-four hours old, injected into abdominal cavity of rat produced death.
94	" "	River	"	0.08	0.52	49.5	1770	Nos. 10 and 31	20 drops of beef-tea culture, twenty-four hours old, injected into abdominal cavity of rat produced death.
95	Torch Lake, Mich.	Lake	"	.....	.....	.....	5281	Nos. 10 and 28	20 drops of beef-tea culture, twenty-four hours old, injected into abdominal cavity of rat caused death.
96	" " "	Well	"	.....	.....	.....	2520	No. 10	Inoculation of a rat produced no effect.

97	Torch Lake, Mich.	Well	First	......	......	......	20	Nos. 10 and 28	Injection of 20 drops of beef-tea culture, twenty-four hours old, into peritoneal cavity killed rat.
98	Pontiac, Mich.	Hydrant	Second	0.422	0.574	9.9	23 after 48 hours	Nos. 10 and 13 and unknown	Inoculations of rats were without effect.
99	" "	Lake	"	0.266	0.302	9.075	18 after 48 hours	No. 10	Inoculation of rat was without effect.
100	" "	"	"	0.130	0.35	9.9	12 after 48 hours	No. 10	" " " " "
101	Negaunee, Mich.	"	First	0.021	0.820	6.105	10	No. 10	" " " " "
102	Parma, Mich	Well	"	0.058	0.121	100.65	1234	No. 10	" " " " "
103	Sault Ste. Marie, Mich.	Lake	Second	0.05	0.104	6.0	5	No. 4	" " " " "
104	" " "	"	"	0.06	0.12	8.25	12	No. 4	" " " " "
105	" " "	"	"	0.176	0.16	11.55	150	Nos. 10 and 4	Inoculations of rats were without effect.
106	Bay City, Mich.	Hydrant	Third	0.366	0.38	339.95	350	No. 10	Inoculation of rat was without effect.
107	Pontiac, Mich.	Well	First	0.198	0.368	41.55	240	No. 13	" " " " "
108	Three Rivers, Mich.	"	Third	0.472	0.212	8.80	26	Nos. 10 and 13	Inoculations of rats were without effect.
109	St. Johns, Mich.	"	First	0.091	0.13	18.15	11,025 after 48 hours	Nos. 13 and 28	1 c.c. of beef-tea culture, twenty-four hours old, was injected into the abdominal cavity of each of three rats; one died, two recovered.
110	" "	"	"	0.13	0.122	18.15	0	Nos. 10, 13, 4	Inoculations of rats were without effect.
111	Duluth, Minn.	Lake	"	0.01	0.121	7.2	200	No. 10	1 c.c. of a culture, forty-eight hours old, was injected into abdominal cavity of rat, results negative.
112	" "	"	"	0.058	0.12	8.25	108	No. 10	1 c.c. of a culture, forty-eight hours old, was injected into abdominal cavity of rat; result negative.
113	" "	"	"	0.007	0.261	8.25	20	Nos. 4, 10, 39	1 c.c. of a culture, forty-eight hours old, was injected into abdominal cavity of rat; result death.
114	" "	"	"	0.038	0.058	9.075	1	No. 10	Inoculation of rat was without effect.
115	" "	Spring	Third	0.058	0.07	14.85	12 after 48 hours	No. 4	" " " " "
116	Leadville, Col.	From a mine	"	0.091	0.091	3.30	145 after 72 hours	Nos. 10 and 4	Inoculations of rats were without effect.
117	Iron Mountain, Mich.	Well	"	0.444	0.441	4.95	5	No. 10	" " " " "
118	" " "	Hydrant	"	0.57	0.57	4.95	6	No. 10	Inoculation of rat was without effect.
119	" " "	Lake	"	0.312	0.312	4.125	2	No. 10	" " " " "
120	" " "	"	Second	0.11	0.322	6.93	5	No 10	" " " " "
121	" " "	"	"	0.092	0.314	7.09	4	No. 10	" " " " "
122	Gaylord, Mich.	Unknown	Third	0.39	0.98	26.40	670 after 48 hours	No. 13	Rats were inoculated without effect.
123	Iron Mountain, Mich.	"	Second	0.03	0.15	4.0	8	No. 13	Injection of 20 drops of beef-tea culture, twenty-four hours old, into abdominal cavity of a rat produced no effect.
124	Norway, Mich.	"	Third	0.01	0.03	1.0	0	Nos. 10 and 13	20 drops of beef-tea culture, twenty-four hours old, injected into the abdominal cavity of a rat produced no effect.
125	" "	"	"	0.02	0.15	3.0	10	Nos. 10 and 13	20 drops of beef-tea culture, twenty-four hours old, injected into the abdominal cavity of a rat produced no effect.
126	" "	Well	"	0.005	0.01	0.5	6	No. 13	Inoculation of rat was without effect.
127	Adrian, Mich.	Unknown	First	0.03	0.20	10.0	40 after 24 hours	Nos. 10 and 28	20 drops of beef-tea, twenty-four hours old, were injected into the abdominal cavity of a rat. Death resulted.

No.	Water sent from	Source.	Class.	Free ammonia.	Albuminoid ammonia.	Chlorine.	Number of germs.	Kind of germs.	Remarks.
128	Iron Mountain, Mich.	Water works	Third	0.005	0.025	2.0	13 after 24 hours	Nos 10 and 21	Rats were inoculated without effect.
129	Pontiac, Mich.	Well	"	0.18	0.10	12.0	30 after 72 hours	Nos. 14 and 21	" " " " "
130	Ironwood, Mich.	River	Second	0.103	0.116	5.6	400 after 24 hours	Nos. 10, 12, 13	" " " " "
131	Iron Mountain, Mich.	Spring	Third	0.07	0.05	0.42	4	Nos. 10, 25, 26	" " " " "
132	Ithaca, Mich.	Unknown	"	0.41	0.14	25.0	500 after 24 hours	Unknown.	20 drops of beef-tea, twenty-four hours old, were injected into the abdominal cavity of a rat without effect.
133	Hastings, Mich.	Well	Second	0.20	0.11	18.0	850	Nos. 14 and 15	Rats were inoculated without effect.
134	Iron Mountain, Mich.	Lake	Third	0.15	0.08	1.3	4	Nos. 10 and 3	" " " " "
135	" " "	Spring	"	0.34	0.106	1.0	2	Nos. 10 and 3	" " " " "
136	Ithaca, Mich.	Well (deep)	"	0.302	0.24	2.8	None	. . . . .	Rat was inoculated without effect.
137	Holland, Mich.	Water works.	Second	0.068	0.09	13.85	20	Nos. 13 and 14	Rats were inoculated with negative results.
138	" "	Well (deep)	"	0.514	0.166	15.675	180 after 48 hours	Nos. 13 and 14	" " " " " "
139	" "	Well (shallow)	"	0.088	0.134	6.93	120 after 48 hours	Nos. 13, 14, 20	" " " " " "
140	Chicago, Ill.	....	......	0.310	0.198	11.55	7	Nos. 9, 10, 24	" " " " " "
141	" "	....	......	0.012	0.06	10.395	90 after 48 hours	Nos. 17, 18, 23	" " " " " "
142	" "	....	......	0.34	0.148	14.85	4	Nos. 2 and 11	" " " " " "
143	" "	....	......	0.178	0.166	8.25	6	Nos. 7, 15, 26	" " " " " "
144	" "	....	......	0.44	0.196	10.98	9	Nos 3 and 27	" " " " " "
145	" "	....	......	0.176	0.106	13.20	48 after 48 hours	Nos. 2, 8, 9, 19, and 27	" " " " " "
146	" "	....	......	0.14	0.106	12.705	6	Nos. 2, 7, 10, 15, 19, 22	" " " " " "
147	" "	....	......	0.48	0.24	18.48	5	Nos. 6, 11, 13, 23	" " " " " "
148	Dunkirk, N. Y.	....	Second	0.091	0.16	2.478	60 after 48 hours	Nos. 10 and 27	" " " " " "

The tubes thus prepared are kept in the incubator for twenty-four hours at 37° in order to see that no contamination with germs from the air has occurred. To the tubes which remain clear, from one to ten drops of the suspected water is added, and the tubes returned to the incubator. If the beef-tea becomes cloudy within the next twenty-four hours, one can, according to Parietti, positively conclude that the typhoid germ is present. The excellence of this test has been attested by Kamen.[1]

Of the seven water germs described in the following tables as growing at 38°, every one grows abundantly in Parietti's solution. Of the twenty-four remaining germs, two were not tested and three were found to grow in Parietti's solution. These three are germs which grow feebly at 38° in ordinary beef-tea, and the addition of Parietti's solution did not further retard their growth.

Uffelmann[2] proposes a test by which many water germs can be excluded in searching for the Eberth germ. His medium consists of a citric acid gelatin, colored with methyl-violet. He states that only a small number of germs, other than the Eberth germ, will grow on this gelatin, and that these do not take up the coloring matter like the Eberth germ. Of the thirty-one water germs upon which I have made this test, nine grow, and some of these stain more intensely than Eberth's germ.

I have used the potato gelatin test of Holz,[3] with and without the addition of carbolic acid as is recommended, with all the toxicogenic germs described in this paper, and I find that all grow quite as readily and abundantly as the Eberth germ.

No. 1. *Micrococcus Candicans.*

Form. Large cocci often grouped in clusters, which vary much in size.
Growth:
  On gelatin plates. Superficial colonies look like minute drops of milk. The deeper ones are more or less brown and granular.
  In gelatin tubes. Grows along the line and forms a "nail head" on the surface.
  On agar. A yellowish or brownish growth.
  On potato. A white, slimy growth.
  In Parietti's solution. Does not grow.
  In Uffelmann's gelatin. Does not grow.
Effect of temperature. Grows at ordinary temperature, but not at 38°.
Rate of growth. Rapid.
Relation to air. Aërobic.
Production of gas. None observed.
Liquefaction. Negative.
Color. White.
Toxicogenic properties. Negative.

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[1] Centralblatt f. Bakteriolgie u. Parasitenkunde, B. xi, No. 2.
[2] Berliner klinische Wochenschrift, 1891, No. 35.
[3] Zeitschrift f. Hygiene, B. viii, S. 143.

No. 2. *Micrococcus Aquatilis Albus.*

Form. Small cocci.
Growth:
  On gelatin plates. Snow-white dots. Spherical colonies with many irregular forms.
  In gelatin tubes. Very slight along the line, and spreading but little over the surface. The growth on the gelatin is not abundant.
  On agar. Very slight, white growth.
  On potato. Thick directly over the place of application, but spreading very little. The color is yellowish-white.
  In Parietti's solution. Does not grow.
  In Uffelmann's gelatin. Does not grow.
Effect of temperature. Grows slowly at ordinary temperature, and more feebly at 38° C.
Rate of growth. Very slow.
Relation to air. Aërobic.
Production of gas. None observed.
Liquefaction. Negative.
Color. White.
Toxicogenic properties. Negative.

No. 3. *Micrococcus Aquatilis Magnus.*

Form. Very large micrococci.
Growth:
  On gelatin plates. Deep brown colonies, with smooth outline.
  In gelatin tubes. Few isolated colonies along the track. Spreads over the surface.
  On agar. White, thin growth.
  On potato. White or brownish granular growth.
  In Parietti's solution. Does not grow.
  In Uffelmann's gelatin. Does not grow.
Effect of temperature. Grows well at ordinary temperature, and feebly at 38° C.
Rate of growth. Moderate.
Relation to air. Aërobic.
Production of gas. None observed.
Liquefaction. Negative.
Color. White.
Toxicogenic properties. Negative.

No. 4. *Micrococcus Luteus.*

Form. Small micrococci, growing in zoöglea.
Growth:
  On gelatin plates. Sulphur-yellow, granular colonies.
  In gelatin tubes. Grows along the line and slowly spreads over the surface.
  On agar. A lemon-yellow growth.
  On potato. An abundant growth, which is at first dirty white, but which gradually becomes lemon-yellow.
  In Parietti's solution. Does not grow.
  In Uffelmann's gelatin. Does not grow.
Effect of temperature. Grows at ordinary temperature, but not at 38° C.
Rate of growth. Rapid.
Relation to air. Aërobic.
Production of gas. None observed.
Liquefaction. Negative.
Color. Lemon-yellow.
Toxicogenic properties. Negative.

No. 5. *Micrococcus Cereus.*

Form. Micrococci.
Growth:
- On gelatin plates. Pale, yellow colonies, of smooth outline.
- In gelatin tubes. Very slight along the track; spreads slowly over the surface.
- On agar. Very faint, white growth.
- On potato. Yellowish white, waxy growth. Beyond the border ot the growth the potato becomes bluish gray.
- In Parietti's solution. Does not grow.
- In Uffelmann's gelatin. Does not grow.

Effect of temperature. Grows at ordinary temperature, but does not grow at 38° C.
Rate of growth. Slow.
Relation to air. Aërobic.
Production of gas. None observed.
Liquefaction. Negative.
Color. White to brownish-yellow.
Toxicogenic properties. Negative.

No. 6. *Micrococcus Subflavus.*

Form. Elliptical micrococci, growing in zoöglea.
Growth:
- On gelatin plates. Yellowish-brown, spherical colonies.
- In gelatin tubes. Very slight along the track; spreads slowly over the surface.
- On agar. White to yellowish-white.
- On potato. Pale, yellow growth, with sloping edges.
- In Parietti's solution. Does grow.
- In Uffelmann's gelatin. Does not grow.

Effect of temperature. Grows at ordinary temperature, and feebly at 38° C.
Rate of growth. Moderate.
Relation to air. Aërobic.
Production of gas. None observed.
Liquefaction. Negative.
Color. Pale yellow.
Toxicogenic properties. Negative.

No. 7. *Micrococcus Aquatilis Invisibilis.*

Form. Oval cocci.
Growth:
- On gelatin plates. Deep brown colonies, with smooth outline; spreading irregularly superficially.
- In gelatin tubes. Slight growth along the line, but spreading on the surface.
- On agar. White, thin growth.
- On potato. Invisible.
- In Parietti's solution. Does grow.
- In Uffelmann's gelatin. Does not grow.

Effect of temperature. Grows well at ordinary temperature, but feebly at 38° C.
Rate of growth. Slow.
Relation to air. Aërobic.
Production of gas. None observed.
Liquefaction. Negative.
Color. White.
Toxicogenic properties. Negative.

No. 8. *Diplococcus Aquatilis.*

Form. Diplococci.
Growth:
On gelatin plates. Smooth, pale yellow colonies, mostly spherical, but showing some irregularities.
On gelatin tubes. Growth confined to surface, where it spreads to the walls of the tube.
On agar. Smooth, white growth.
On potato. Dirty white and glazed; thick, with sloping borders.
In Parietti's solution. Does not grow.
In Uffelmann's gelatin. Does not grow.
Effect of temperature. Does not grow at 38°; grows well at ordinary temperature.
Rate of growth. Moderate.
Relation to air. Aërobic.
Production of gas. None.
Liquefaction. None.
Color. White.
Toxicogenic properties. Negative.

No. 9. *Streptococcus Aquatilis.*

Form. Large micrococci; some single, others in pairs, and some in chains of from four to eight.
Growth:
On gelatin plates. Colonies spherical, many having notched outlines.
In gelatin tubes. Grows very slightly along the line, but spreads over the surface to the walls of the tube.
On agar. Very thin, white growth.
On potato. Heavy, moist, white growth.
In Parietti's solution. Does not grow.
In Uffelmann's gelatin. Does not grow.
Effect of temperature. Does not grow at 38°; grows at ordinary temperature.
Rate of growth. Slow on gelatin; rapid on potato.
Relation to air. Aërobic.
Production of gas. None.
Liquefaction. Negative.
Color. White.
Toxicogenic properties. Negative.

No. 10. *Bacillus Fluorescens Liquefaciens.*[1]

Form. Very small bacilli; twice as long as broad.
Growth:
On gelatin plates. Small, round colonies, liquefying and sinking into the gelatin.
In gelatin tubes. Liquefies gelatin rapidly from surface evenly to bottom; no scum on surface; deposit heavy and yellowish white. The supernatant fluid is yellowish, with green fluorescence, most marked near the surface.
On agar. Thin white growth.
On potato. Smooth growth, with yellowish tint and sloping margins.
In Parietti's solution. Does not grow.
In Uffelmann's gelatin. Does not grow.

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[1] I have found two varieties of this germ. The second differs from the one described in this table in the following particulars: 1. In gelatin tubes the liquefaction proceeds slowly, and after half or two-thirds of the gelatin has been liquefied, ceases altogether; the subjacent gelatin showing marked fluorescence. 2. It grows abundantly in Uffelmann's gelatin, and takes the stain more markedly than does Eberth's germ.

Effect of temperature. Grows at ordinary temperature, but does not grow at 38° C.
Rate of growth. Rapid at ordinary temperature.
Relation to air. Aërobic.
Production of gas. None observed.
Liquefaction. Liquefies rapidly and completely.
Color. Yellowish white.
Toxicogenic properties. Negative.

No. 11. *Bacillus Fluorescens Non-liquefaciens.*

Form. Small bacilli, with rounded ends.
Motility. Non-motile.
Growth:
- On gelatin plates. Glistening, mother-of-pearl colonies, spreading on the surface.
- In gelatin tubes. Growth confined to surface, and dirty yellow in color; subjacent gelatin shows green fluorescence.
- On agar. White on the surface, while the mass of agar becomes green.
- On potato. Grayish white or brown growth, with glistening surface.
- In Parietti's solution. Does not grow.
- In Uffelmann's gelatin. Does not grow.

Effect of temperature. Grows well at ordinary temperature, but does not grow at 38° C.
Rate of growth. Rapid at ordinary temperature.
Relation to air. Aërobic.
Production of gas. None observed.
Liquefaction. Non-liquefying.
Color. White, or yellowish to brownish white.
Toxicogenic properties. Negative.

No. 12. *Bacillus Gasoformans.*

Form. Small bacilli, two to three times as long as broad.
Motility. Motile.
Growth:
- On gelatine plates. Large colonies, which rapidly liquefy the gelatin.
- In gelatin tubes. Rapidly liquefies the gelatin, and bubbles of gas form along the line of inoculation.
- On agar. A brownish or yellowish growth.
- On potato. Dirty white growth.
- In Parietti's solution. Does not grow.
- In Uffelmann's gelatin. Does not grow.

Effect of temperature. Grows at ordinary temperature, but not at 38° C.
Rate of growth. Rapid.
Relation to air. Aërobic.
Production of gas. Produces gas abundantly.
Liquefaction. Liquefies gelatin rapidly.
Color. White.
Toxicogenic properties. Negative.

No. 13. *Bacillus Liquefaciens Albus.*

Form. Small rod, with rounded ends.
Motility. Very motile.
Growth:
- On gelatin plates. Small, round colonies, liquefying rapidly.
- In gelatin tubes. Grows rapidly, and completely liquefies the gelatin.

On agar. A dirty white growth.
On potato. Yellowish or brownish white.
In Parietti's solution. Does not grow.
In Uffelmann's gelatin. Does not grow.
Effect of temperature. Does not grow at 38°; grows well at ordinary temperature.
Rate of growth. Moderate.
Relation to air. Aërobic.
Production of gas. The gelatin tubes give off no odor, and for this reason this germ must not be confounded with the bacillus liquefaciens, as described by Eisenberg.
Liquefaction. Liquefies rapidly.
Color. White.
Toxicogenic properties. Negative.

No. 14. *Bacillus Albus.*

Form. Short bacilli, often in chains.
Motility. Motile.
Growth:
On gelatin plates. White, smooth colonies.
In gelatin tubes. Grows slowly along the line of inoculation, and forms a white "nail head" on the surface.
On agar. A milk-white growth.
On potato. A dirty yellowish-white growth.
In Parietti's solution. Does not grow.
In Uffelmann's gelatin. Does not grow.
Effect of temperature. Grows at ordinary temperature, but does not grow at 38° C.
Rate of growth. Slow.
Relation to air. Aërobic.
Production of gas. None observed.
Liquefaction. Does not liquefy.
Color. White.
Toxicogenic properties. Negative.

No. 15. *Bacillus Albus Putridus.*

Form. Long and short bacilli, often growing in chains.
Motility. Motile.
Growth:
On gelatin plates. Round, white or brown colonies.
In gelatin tubes. Growth confined to surface, where it becomes very heavy. Liquefaction extends downward very slowly. (In these respects this germ differs from that described by Eisenberg under this name.) The contents of the tube give off a highly offensive odor.
On agar. Heavy, smooth growth.
On potato. Slimy, grayish-white; later, brownish growth.
In Parietti's solution. Does not grow.
In Uffelmann's gelatin. Does not grow.
Effect of temperature. Does not grow at 38°; grows well at ordinary temperature.
Rate of growth. Rapid.
Relation to air. Aërobic.
Production of gas. Gives off putrefactive odors.
Liquefaction. Liquefies slowly.
Color. White, or yellowish white.
Toxicogenic properties. Negative.

No. 16. *Bacillus Violaceus.*

Form. Bacilli about three times as long as broad, often growing in threads.
Motility. Motile.
Growth:
  On gelatin plates. The colonies look like small air bubbles to the unaided eye. Under the microscope the colonies are granular and violet. Sometimes the surface of the gelatin is raised like a foam.
  In gelatin tubes. Produces a funnel-shaped liquefaction, while the pale violet mass of germs lies at the bottom.
  On agar. A blue to violet growth.
  On potato. A dark violet growth.
  In Parietti's solution. Not tested.
  In Uffelmann's gelatin. Not tested.
Effect of temperature. Grows at ordinary temperature, but not at 38° C.
Rate of growth. Moderate.
Relation to air. Facultative anaërobic.
Production of gas. None observed.
Liquefaction. Liquefies.
Color. Produces a violet color.
Toxicogenic properties. Negative.

No. 17. *Bacillus Gracilis Aërobicus.*

Form. Bacilli four to six times as long as broad.
Motility. Motile.
Growth:
  On gelatin plates. Small, yellow colonies of rapid growth.
  In gelatin tubes. Grows abundantly along the track.
  On agar. Grayish-white, feeble growth.
  On potato. A waxy growth of pinkish-white color, while beyond the growth the potato becomes steel gray.
  In Parietti's solution. Does not grow.
  In Uffelmann's gelatin. Grows abundantly, and stains more intensely than does Eberth's germ.
Effect of temperature. Grows at ordinary temperature; does not grow at 38°.
Rate of growth. Rapid.
Relation to air. Aërobic.
Production of gas. None observed.
Liquefaction. Negative.
Color. White to gray.
Toxicogenic properties. Negative.

No. 18. *Bacillus Gracilis Anaërobiescens.*

Form. Bacilli three times as long as broad, often growing in long, slender rods.
Motility. Very motile.
Growth:
  On gelatin plates. Brownish colonies, spreading irregularly.
  In gelatin tubes. Grows abundantly along the line, and also spreads over the surface.
  On agar. Thin, white growth.
  On potato. Growth is yellowish white, abundant and prominent. Stains made from potato show long threads, with shorter bacilli near, often presenting the appearance of fringe.
  In Parietti's solution. Grows.
  In Uffelmann's gelatin. Does not grow.

Effect of temperature. Grows well at ordinary temperature, and feebly at 38°.
Rate of growth. Rapid.
Relation to air. Grows under gelatin.
Production of gas. Forms gas abundantly when grown under gelatin.
Liquefaction. Negative.
Color. White.
Toxicogenic properties. Negative.

No. 19. *Bacillus Helvolus.*

Form. Short bacilli, often growing in chains, and frequently forming long threads.
Motility. Only an oscillation.
Growth:
  On gelatin plates. Small, yellowish or brownish colonies.
  In gelatin tubes. Forms on the surface a spreading white growth, which later becomes yellow. The growth spreads to the walls of the tube, and very slowly sinks in the gelatine as liquefaction goes on. The growth along the line is very slight, or wanting altogether.
  On agar. Yellowish white growth.
  On potato. Yellow.
  In Parietti's solution. Does not grow.
  In Uffelmann's gelatin. Does not grow.
Effect of temperature. Grows well at ordinary temperature, but only feebly at 38°.
Rate of growth, Slow.
Relation to air. Aërobic.
Production of gas. None observed.
Liquefaction. Liquefies very slowly.
Color. White to yellow.
Toxicogenic properties. Negative.

No. 20. *Bacillus Rubidus.*

Form. A bacillus four to six times as long as broad.
Motility. Sluggishly motile.
Growth:
  On gelatin plates. Yellowish, irregular colonies.
  In gelatin tubes. Slight growth of isolated colonies along the line; these soon become pink in color. The growth at the surface is abundant, and as the gelatin liquefies large masses of the germ, with faint pink tint along the lower border, float in the liquid.
  On agar. Faint yellowish growth, with no evidence of the possible development of color shown on some other media.
  On potato. The growth has at first a slight pinkish tint, which, with age, develops into a beautiful red.
  In Parietti's solution. Does not grow.
  In Uffelmann's gelatin. Does not grow.
Effect of temperature. Grows well at ordinary temperature, but does not grow at 38° C.
Rate of growth. Moderate.
Relation to air. Aërobic.
Production of gas. None observed.
Liquefaction. Liquefies slowly.
Color. Red.
Toxicogenic properties. Negative.

No. 21. *Bacillus Cinnabareus.*

Form. A short bacillus.
Motility. Highly motile.
Growth:
  On gelatin plates. Brick-red colonies.
  In gelatin tubes. Liquefies rapidly and completely.
  On agar. Cinnabar-red growth.
  On potato. An abundant growth, at first yellowish-white, becoming more red with age.
  In Parietti's solution. Not tested.
  In Uffelmann's gelatin. Does not grow.
Effect of temperature. Grows at ordinary temperature; does not grow at 38° C.
Rate of growth. Rapid.
Relation to air. Not tested.
Production of gas. None observed.
Liquefaction. Liquefies rapidly.
Color. Cinnabar-red.
Toxicogenic properties. Negative.

No. 22. *Bacillus Subflavus.*

Form. Bacilli two to three times as long as broad.
Motility. Motile.
Growth:
  On gelatin plates. Small, glistening, white, irregular colonies.
  On gelatin tubes. Grows but slightly along the track; spreads over the surface. The tube shows slight fluorescence.
  On agar. Yellowish-white growth; with age the mass of agar becomes brownish-yellow.
  On potato. A beautiful salmon-colored granular growth.
  In Parietti's solution. Does not grow.
  In Uffelmann's gelatin. Grows abundantly, and stains more intensely than Eberth's germ.
Effect of temperature. Grows at ordinary temperature; does not grow at 38° C.
Rate of growth. Rapid.
Relation to air. Aërobic.
Production of gas. None observed.
Liquefaction. Negative.
Color. Yellowish-white.
Toxicogenic properties. Negative.

No. 23. *Bacillus Ochraceus.*

Form. Bacilli two to three times as long as broad, with rounded ends.
Motility. Non-motile. (In which respect it differs from Zimmermann's germ of the same name.)
Growth:
  On gelatin plates. Small, yellow, irregular colonies, spreading slightly over the surface.
  In gelatin tubes. Slowly liquefies evenly from the surface; the mass of germ lying on the bottom of the liquefied part, and showing at first a pale yellow, then an orange growth.
  On agar. Forms an even yellow growth.
  On potato. A beautiful and abundant growth of ochre yellow. This growth is very thick and heavy, and in this respect differs from the germ of Zimmermann.
  In Parietti's solution. Does not grow.
  In Uffelmann's gelatin. Does not grow.

Effect of temperature. Does not grow at 38°; grows well at ordinary temperature.
Rate of growth. Moderate.
Relation to air. Aërobic.
Production of gas. None.
Liquefaction. Liquefies slowly.
Color. Orange to ochre.
Toxicogenic properties. Negative.

No. 24. *Bacillus Figurans.*

Form. Bacilli two to three times as long as broad, but showing marked variation in form. Sometimes they appear as very short bacilli, while at other times they grow into long threads.
Motility. Sluggishly motile.
Growth:
- On gelatin plates. The deep colonies are spherical and smooth, but the superficial growth forms curved and interlacing lines, often presenting most grotesque figures. Plates may show no liquefaction after some days.
- In gelatin tubes. Does not develop along the line. Generally liquefies from the surface before the growth has reached the walls of the tube. Liquefaction, however, goes on very slowly, and, in some instances, the fluid is lost by evaporation as fast as it liquefies, and consequently there is apparently no liquefaction. After the gelatin has been liquefied half way down the tube, the mass of germs subsides, and further liquefaction is very slow or does not occur at all.
- On agar. Forms a thin white growth, with heavy deposit often in water of condensation.
- On potato. Abundant, faintly yellow, mucilaginous growth, without raised edges.
- In Parietti's solution. Does not grow.
- In Uffelmann's gelatin. Does not grow.

Effect of temperature. Grows well at ordinary temperature; feebly at 38° C.
Rate of growth. Very rapid at ordinary temperature.
Production of gas. None observed.
Liquefaction. Liquefies half-way down the tube.
Color. White.
Toxicogenic properties. Negative.

No. 25. *Micrococcus Aquatilis.*

Form. Small cocci, often in groups.
Growth:
- On gelatin plates. Small, round, white colonies.
- In gelatin tubes. Very feeble growth along the line, but spreading over the surface.
- On agar. White, thin growth.
- On potato. Brownish, moist growth.
- In Parietti's solution. Grows.
- In Uffelmann's gelatin. Does not grow.

Effect of temperature. Grows at ordinary temperature, also at 38° C.
Rate of growth. Slow.
Relation to air. Aërobic.
Production of gas. None observed.
Liquefaction. Negative.
Color. White.
Toxicogenic properties. Negative.

No. 26. *Bacillus Albus Anaërobiescens.*

Form. Bacilli, two to three times as long as broad.
Motility. Only an oscillation.
Growth:
- On gelatin plates. Smooth, spherical, yellowish or brownish colonies.
- In gelatin tubes. Grows well along the track, and spreads over the surface.
- On agar. Heavy, milk-white growth.
- On potato. Yellowish-white, glistening growth.
- In Parietti's solution. Grows.
- In Uffelmann's gelatine. Grows, and stains with the same intensity as Eberth's germ.

Effect of temperature. Grows at ordinary temperature, and also at 38° C.
Rate of growth. Rapid.
Relation to air. Grows under gelatin.
Production of gas. None observed.
Liquefaction. Does not liquefy.
Color. White.
Toxicogenic properties. Negative.

No. 27. *Bacillus Invisibilis.*

Form. Large bacilli, from two to five times as long as broad, with rounded ends.
Motility. Motile.
Growth:
- On gelatin plates. Pale yellow burr-like colonies, with irregular outline, and spreading slightly.
- In gelatin tubes. Grows abundantly along the track, and spreads slowly over the surface.
- On agar. Thick white growth, with but little tendency to spread.
- On potato. Invisible growth.
- In Parietti's solution. Grows.
- In Uffelmann's gelatin. Grows abundantly, but does not take up the stain.

Effect of temperature. Grows at ordinary temperature, and at 38°.
Rate of growth. Rapid.
Relation to air. Grows under gelatin.
Production of gas. None observed.
Liquefaction. Does not liquefy.
Color. White.
Toxicogenic properties. Negative.

No. 28. *Bacillus Venenosus.*

Form. A bacillus from two to four times as long as broad, with rounded ends.
Motility. Very motile.
Growth:
- On gelatin plates. Small, white dots, spherical, and sometimes slightly yellow. The superficial colonies are raised above the surface of the gelatin.
- In gelatin tubes. Grows abundantly along the line, and slowly spreads over the surface. When taken from the spleen of an animal which has died twelve days or later after inoculation, the tendency to spread over the surface of the gelatin is less marked than with the germ which has not passed through the animal body.
- On agar. A thin white growth.

On potato. A light brown, moist growth. When the germ is taken from the spleen of an inoculated animal the growth is often invisible.
In Parietti's solution. Grows abundantly.
In Uffelmann's gelatin. Grows abundantly, and the superficial colonies stain intensely, while the deeper ones are not so markedly stained as those of the Eberth germ.
Effect of temperature. Grows rapidly both at ordinary temperature and at 38° C.
Rate of growth. Rapid.
Relation to air. Grows well under gelatin.
Production of gas. None observed.
Liquefaction. Does not liquefy.
Color. White.
Toxicogenic properties. Toxicogenic in rats, mice, guinea-pigs, and rabbits. See experimental evidence on this point given later.

No. 29. *Bacillus Venenosus Brevis.*

Form. A thick, short bacillus; length about twice the width; grows in threads in old cultures.
Motility. Motile.
Growth:
On gelatin plates. Small, round colonies, with concentric rings. Generally, the deeper colonies are yellowish or brown. The surface colonies are raised, and spread but little. On plates made from the spleen of an inoculated animal the colonies are less regular in outline, and show a marked tendency to spread over the surface.
In gelatin tubes. Grows well along the line and spreads slowly over the surface, but finally extends to the sides of the tube.
On agar. Thin, white growth.
On potato. Moist, light brown, thick growth. Cultures which have been kept for fourteen days or longer at 40° form an invisible growth on potato. The medium used in these experiments was fresh spleen from man.
In Parietti's solution. Grows abundantly.
In Uffelmann's gelatin. Grows slowly, and takes the stain less markedly than does Eberth's germ.
Effect of temperature. Grows both at ordinary temperature and at 38° C.
Rate of growth. Rapid.
Relation to air. Grows well under gelatin.
Production of gas. None observed.
Liquefaction. Does not liquefy.
Color. White.
Toxicogenic properties. Toxicogenic in rats, mice, and guinea-pigs, and rabbits.

No. 30. *Bacillus Venenosus Invisibilis.*

Form. A slender bacillus; length, from two to four times the breadth. Ends are rounded.
Motility. Motile.
Growth:
On gelatin plates. Colonies are small, yellowish, and granular. Surface colonies are very irregular in shape and size, and are coarsely granular.
In gelatin tubes. Grows very slowly both along the line and at the surface; scarcely visible after three days.
On agar. A very thin, white growth.

On potato. On some potatoes the growth is invisible; on others there is a light brown growth.
In Parietti's solution. Grows well.
In Uffelmann's gelatin. Grows well and stains.
Effect of temperature. Grows at ordinary temperature and at 38°.
Rate of growth. Slow.
Production of gas. None observed.
Liquefaction. Does not liquefy.
Color. White.
Toxicogenic properties. Toxicogenic, but in less degree than bacillus venenosus. (See inoculation experiments.)

No. 31. *Bacillus Venenosus Liquefaciens.*

Form. A bacillus, whose length is from one and one-half to two times its breadth, and which has rounded ends.
Motility. Motile.
Growth:
On gelatin plates. The superficial colonies are raised, and spread over the surface. The deep colonies are yellowish and finely granular, with unbroken outline.
In gelatin tubes. Grows along the line abundantly, and spreads over the surface slowly. Liquefaction begins after from four to six weeks.
On agar. A thin, white growth.
On potato. A moist, light brown or yellowish growth. When kept for fourteen days or longer on spleen tissue, it forms an invisible growth on potato.
In Parietti's solution. Grows abundantly.
In Uffelmann's gelatin. Grows abundantly and stains deeply.
Effect of temperature. Grows at ordinary temperature and at 38°.
Rate of growth. Rapid.
Relation to air. Grows under gelatin.
Production of gas. Gas bubbles occasionally appear in gelatin tubes.
Liquefaction. Liquefies gelatin after some weeks.
Color. White.
Toxicogenic properties. Toxicogenic to mice, rats, guinea-pigs, and rabbits. (See inoculation experiments.)

The effects of the toxicogenic germs which I have described are compared with those of the Koch-Eberth germ. None of these can be said to be highly toxicogenic to the lower animals. They are not comparable with the germs of anthrax, glanders, tetanus, or diphtheria. I have made a great many inoculation experiments with these germs, but will detail only a few which I have made recently. In my inoculation experiments with Eberth's bacillus, I have used a germ obtained by myself from the spleen of a person dead from typhoid fever, and a germ obtained from the Berlin Hygienic Laboratory. The two sets of inoculations are detailed separately.

RESULTS OF INOCULATIONS.

BACILLUS VENENOSUS.

Experiments 1–5.—Five white rats were inoculated by the injection of two cubic centimetres of an alkaline nuclein[1] culture, twenty-six days old, into the abdominal cavity. All died within twenty-four hours. The spleen, kidneys, and liver were found engorged. Plates made from these organs showed abundant growth of the germ in pure culture. Five cubic centimetres of this culture, after being freed from germs by filtration through porcelain, were injected into the abdominal cavities of rats without effect.

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[1] The preparation of this medium will be described in a later paper on the chemical products of these germs.

Exp. 6–20.—Fifteen white rats were inoculated by the injection of two cubic centimetres of a beef-tea culture, grown for twenty-four hours at a temperature of 38°, into the abdominal cavity. All died within twenty-four hours. The spleen, kidneys, and liver were engorged. Plates made from these organs gave positive results.

Exp. 21–26.—Five white rats were inoculated by the injection into the abdominal cavity of one cubic centimetre of a beef-tea culture, grown for twenty-four hours at 38°. All died within twenty-four hours. Results of the examination and of the plates were the same as those given above. Six cubic centimetres of this culture, after being freed from germs by filtration through porcelain, were injected into the abdominal cavity of rats without effect.

Exp. 27–29.—Three white mice were inoculated by the subcutaneous injection of one cubic centimetre each of nuclein culture six days old. The inoculations were made at 4 P. M. Up to 9 P. M. the animals were apparently unaffected. The next morning they were found with eyes closed, rough coat, refusing to eat, and lying in a stupid state. They remained in this condition throughout the day, and were found dead the next morning, about forty hours after being inoculated. All of these mice suffered from diarrhœa before death. Post-mortem examination showed the intestines containing yellowish, watery feces. No marked changes were observed. Plates were made from the spleen, kidney, and liver of each. All these plates showed pure cultures of the germ.

Exp. 30. A guinea-pig was given two cubic centimetres of a nuclein culture six days old, under the skin of the back. The animal died after forty-eight hours. Examination showed the spleen slightly enlarged, and the intestines filled with watery feces. The animal had marked diarrhœa the day before it died. Plates were made from the spleen, tube cultures from the plates, and potato cultures from the tube. The growths on the potatoes were all less marked than those on companion potatoes made from the tubes of the germ which had not been through an animal.

Exp. 31–33.—Three white rats were given each one cubic centimetre of nuclein culture, six days old, under the skin of the back. The next day all had rough coats and all refused to eat. On the third day after the inoculation one of the rats died. Post-mortem showed the intestines inflamed, and the surface of the liver marked with many white nodules, each of which was about an eighth of an inch in diameter. These spots contained pure cultures of the germ. The spleen, which was engorged, also yielded the germ in large numbers. One of the rats seemed to gradually improve and on the twentieth day it was killed and the kidney, spleen, and liver plated. These plates remained sterile. The third rat developed three large, lymphatic tumors, one in each groin and one in the left axilla. The animal ate its food but still retained its rough coat and became very much emaciated. On the thirtieth day after the inoculation the animal was killed and plates were made from one of these tumors and from the liver, spleen, and kidney. The only abnormality observed in the abdominal organs was the enlargement of the spleen, which was twice as large as normal. A small number of colonies appeared on the plates made from the spleen, while the other plates remained sterile. Tubes were made from the spleen plates and the identity of the germ fully determined. This shows that this bacillus may live in the body of the rat for thirty days. This result I regard as exceptional, and due to the condition of the animal more than to the virulence of the germ.

Exp. 34 and 35.—Injected under the skin of the back of each of two gray mice one cubic centimetre of a twenty-four hours' beef-tea culture. The next day these animals remained in a lethargic condition, and on the following day they died. There was marked congestion of the bloodvessels of the mesentery and, in one, fecal matter was found in the peritoneal cavity. The spleen, liver, and kidney were dark. Plates made from these organs developed the germ.

Exp. 36 and 37.—Injected under the skin of the back of each of two guinea-pigs two cubic centimetres of a twenty-four hours' beef-tea culture; both had rough coats and ate but little during the next two days. After this one rapidly improved and was soon apparently well. The other gradually lost flesh and died on the twelfth day after inoculation. The only abnormality which could be detected was an enlargement of the spleen, which was at least four times the size of this organ in healthy guinea-pigs. From the spleen, liver, and kidney cultures were made, and on each plate a small number of colonies of the germ appeared. Tube and potato cultures were made from these plates, and a wholly invisible growth formed on the potatoes.

Exp. 38–40.—Inoculated three white rabbits by the injection of three cubic centimetres of an alkaline nuclein culture under the skin of the back. Two of the animals were, so far as could be seen, not affected. The third began, on the second day, to show evidence of a profuse diarrhœa, which continued and was accompanied by great emaciation. The animal continued to eat, and, indeed, it ate more than either of the others, and seemed to have an insatiable appetite. The diarrhœa and emaciation continued for two weeks, and at the expiration of this time it was much reduced, but it continued to eat and soon began to regain flesh. After two weeks more it showed but slight evidence of the previous emaciation.

Exp. 41.—Injected two cubic centimetres of a nuclein culture into the abdominal cavity of a white rabbit about eight weeks old. The next morning the animal had a profuse diarrhœa, and died within forty-eight hours after the inoculation. The spleen was much congested and the small intestines highly inflamed. Plates made from the spleen, liver, and kidney showed abundant germs.

Exp. 42.—Injected two and one-half cubic centimetres of beef-tea culture, seven days old, under the skin on the back of a guinea-pig which had survived a previous inoculation with Eberth's germ. The animal was not affected, so far as could be seen.

BACILLUS VENENOSUS BREVIS.

Exp. 1 and 2.—Injected three cubic centimetres of a beef-tea culture, twenty-four hours old, into the abdominal cavity of each of two large rabbits. Both animals died within thirty hours. The spleen, liver, and mesentery were much congested. Cultures made from the spleen, liver, and kidney showed numerous colonies of the germ.

Exp. 3.—Injected two cubic centimetres of a beef-tea culture into the abdominal cavity of a large rabbit. The animal died within twenty-four hours. The post-mortem appearance and the cultures corresponded with those in the preceding experiments.

Exp. 4.—Injected one and one-half cubic centimetres of a beef-tea culture, twenty-four hours old, into the peritoneal cavity of a large rabbit. The animal rapidly emaciated, and the temperature in the rectum was taken twice a day from the eighth to the seventeenth day after inoculation, and was found to vary from 103° to 104.4°. During this time there was profuse diarrhœa. On the eighteenth day the animal was so weak that it could not stand and it was chloroformed and examined. The following observations made at the examination are given:

1. There was no evidence of suppuration at the point of inoculation or elsewhere.
2. The lower four inches of the small intestines were dark and congested externally, and the lower two inches were entirely denuded of epithelium.
3. The intestines contained a yellowish, frothy fluid.
4. The spleen, liver, and kidney were enlarged and somewhat hardened.
5. Cultures from these organs gave pure cultures of the germ.

Exp. 5.—Fifteen drops of a beef-tea culture were injected into the abdominal cavity of a rat; the animal was found dead the next morning. The spleen, liver, and kidney were congested and cultures from these organs gave positive results.

Exp. 6–10.—Two cubic centimetres of a beef-tea culture were injected under the skin on the back of each of five guinea-pigs. All died within from two to ten days after the inoculation. In all the spleen was found enlarged and cultures from this organ gave positive results

Exp. 11 and 12.—One cubic centimetre of a beef-tea culture was injected under the skin on the back of each of two white mice; the animals died within forty-eight hours and cultures from spleen, liver, and kidney showed abundant colonies of the germ.

BACILLUS VENENOSUS INVISIBILIS.

Exp. 1–10.—Injected one cubic centimetre of a beef-tea culture into the abdominal cavity of each of ten white rats. All died within forty-hours. The spleen, liver, and kidney were congested and cultures from these organs gave positive results.

Exp. 11–14.—Injected one cubic centimetre of a beef-tea culture, twenty-four hours old, under the skin of the back of each of four white mice. All died on the second day. Cultures from the spleen, liver, and kidney gave positive results.

Exp. 15 and 16.—Injected one and one-half cubic centimetres of a nuclein culture under the skin on the back of each of two guinea-pigs; the animals were not affected.

Exp. 17.—Injected one cubic centimetre of a beef-tea culture under the skin on the back of gray mouse. The animal died within twelve hours and cultures were made from the spleen, liver, and kidney with positive results.

BACILLUS VENENOSUS LIQUEFACIENS.

Exp. 1–3.—Injected one cubic centimetre of a beef-tea culture, twenty-four hours old, under the skin on the back of each of three gray mice. One died within eighteen hours, a second within thirty hours, and the fourth within forty-eight hours. Post-mortem examination in each showed marked engorgement of the mesentery and the spleen. Plates revealed the presence of the germ.

Exp. 4–12.—Injected fifteen drops of a beef-tea culture into the abdominal cavity of each of ten white rats. All died within twenty-four hours. Examinations showed the spleen markedly engorged and the liver and kidney slightly so. Plates from these organs gave positive results. Five cubic centimetres of this culture, after filtration through porcelain, were injected into the abdominal cavity of each of five rats without causing death.

Exp. 13.—Injected three cubic centimetres of a beef-tea culture, seventy-two hours old, into the abdominal cavity of a rabbit. The animal died within twenty-four hours. The spleen was found engorged and cultures showed the presence of the germ.

EBERTH'S GERM (*from spleen*).

Exp. 1.—Injected three cubic centimetres of a beef-tea culture, twenty-four hours old, into the abdominal cavity of a rabbit. The animal died within twenty-four hours.

Exp. 2.—Same as No. 1, except that the animal remained apparently unaffected until the eleventh day, when it was found to be completely paralyzed in the posterior extremities. Two days later the rabbit died. Examination showed the mesenteric glands very much enlarged. The mucous membrane of the small intestine was congested; Peyer's patches enlarged; externally the lower end of the small intestine was covered with a plastic exudate. Cultures were made from kidney, liver, spleen, and blood, but all the plates remained sterile.

Exp. 3–5.—Injected one cubic centimetre of a beef-tea culture, twenty-four hours old, into the abdominal cavity of each of three white rats. One died after forty hours. The mucous membrane of the lower portion of the small intestine was markedly inflamed. Cultures gave positive results. The other rats were not seriously affected.

Exp. 6–8.—Injected into the abdominal cavity of three white rats, respectively, fifteen, ten, and five drops of a beef-tea culture, twenty-four hours old. The one which had ten drops died within twenty-four hours. The post-mortem appearances were like those of the rat which had one cubic centimetre. The other rats were not affected.

Exp. 9–11.—Injected two cubic centimetres of a beef-tea culture into the abdominal cavity of each of three white rats. Two died within twenty-four hours, in each the lower portion of the small intestine was inflamed internally and covered with a plastic exudate externally. Plates made from the liver, spleen, and kidney, gave positive results. The third rat recovered.

Exp. 12.—Injected one-half cubic centimetre into the abdominal cavity of a white rat. The animal did not appear to be seriously affected. It was killed on the fourth day. The spleen was about three times the normal size. Cultures gave numerous colonies.

Exp. 13.—Injected one cubic centimetre of a beef-tea culture, twenty-four hours old, into the abdominal cavity of a half-grown rabbit. The animal died within twenty-six hours. The mucous membrane of the lower portion of the small intestine was slightly inflamed. No other changes were observed. Plates showed numerous colonies.

Exp. 14.—Injected into the abdominal cavity of a guinea-pig one cubic centimetre of a beef-tea culture, twenty-four hours old. The animal died within twenty-four hours. There was slight redness of the small intestine. Cultures gave positive results.

EBERTH'S BACILLUS (*from Berlin*).

Exp. 1 and 2.—Injected one cubic centimetre of a beef-tea culture, twenty-four hours old, into the abdominal cavity of each of two white rats. One died within twenty-four hours. The lower portion of the small intestine was inflamed, and was covered externally with a plastic exudate. The second rat did not die, but was killed on the fourth day. The spleen was enlarged, but not so markedly as in the rat which had been inoculated with the bacillus from the spleen.

Exp. 3 and 4.—Injected two cubic centimetres of a beef-tea culture, twenty-four hours old, into the abdominal cavity of each of two white rats. One died within twelve hours. The spleen, liver, and kidney were engorged. Plates were successful. The second rat did not appear to be affected by the inoculation.

Relation of these Toxicogenic Germs to the Bacillus of Eberth.

From the studies which I have detailed, the following points seem to me to be demonstrated:

1. In their effects upon the lower animals these toxicogenic germs are fully equal in virulence to the Eberth bacillus.

2. The post-mortem appearances in animals dying after inoculation with these germs, are practically identical with those observed in animals killed by inoculation with the Eberth germ.

3. While the bacillus venenosus, as taken from water, forms on potato

a growth wholly different from that of the Eberth bacillus, the former, when taken from animals which have survived the inoculation for twelve days or longer, forms on the potato the invisible growth which is generally supposed by bacteriologists to be characteristic of the Eberth bacillus.

4. Two of these toxicogenic germs form on potatoes invisible growths, when taken from cultures in human spleen which have been kept at a fever temperature for some days.

5. The fourth toxicogenic germ, when taken directly from the water, forms an invisible growth on potato.

If we add to these the fact, well known to bacteriologists, that there are marked morphological and culture differences between Eberth germs from spleens in different epidemics of typhoid fever, and that the most skilful bacteriologists have reported most diversely upon the reaction of the Eberth germ with staining reagents, the evidence becomes sufficient to convince me that the Eberth germ, as found in the spleens and other organs after death, is not a specific microörganism, but is a modified or involution form of any one of a number of related germs. (I have refrained from discussing the reaction of the Eberth germ with staining reagents in this paper because I have elsewhere gone quite thoroughly into this point of controversy.)

If we cannot agree in this conclusion, we must accept the view of Babes, that there are varieties of the Eberth bacillus. It probably makes but little difference whether we conclude that these germs are varieties of one species or that they are related species. I know of no hard and fast lines upon which one can decide, to the satisfaction of everyone else, whether two or more germs, differing more or less, should be classified as species or as varieties.

Of one thing I am certain, and that is that I am ignorant of any crucial test, or of any combination of tests, upon the strength of which I can say at present that a germ which I may find in drinking-water is identical with the so-called typhoid bacillus. I have found in spleens after death from typhoid fever, germs which differ from the typhoid bacillus obtained from Berlin, and from one another as markedly as my bacillus venenosus differs from either or both.